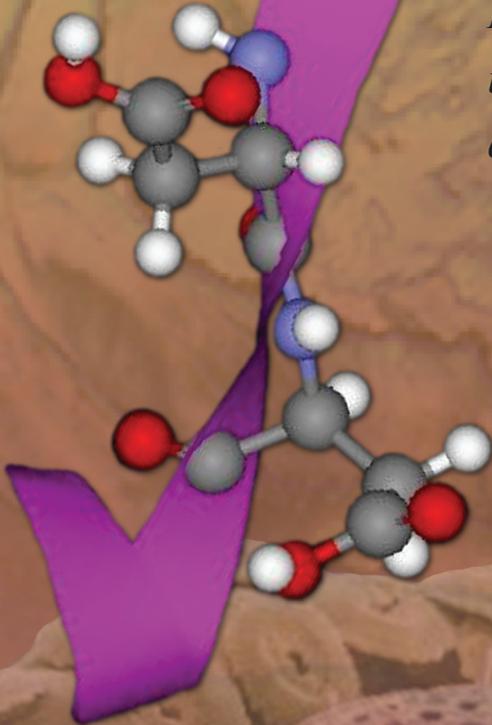


Mimicking Nature's Crystalline Structures

Nature's biominerals hold the key to designing unique nanostructures for human health and industrial applications.



SCIENTISTS and engineers have long envied nature's ability to design crystalline structures whose properties are often superior to those of similar synthetic materials. Through a process called biomineralization, proteins orchestrate the growth processes of many natural minerals into designs that confer exceptional properties. Scientists are eager to understand nature's biomineralization processes. The biological controls that determine the size, shape, and properties of crystals are key to addressing challenges as diverse as synthesizing nanostructures, characterizing climate change, treating disease, and designing new materials for national security applications.

Researchers have been studying biominerals for decades and have known for some time that organic molecules can influence the shape and properties of a growing crystal. However, researchers have been limited in their ability to mimic biomineral growth for specific applications, because they lack a thorough understanding of how biominerals form. The limitations are evident by comparing a single calcite crystal (the most stable form of calcium carbonate) synthesized in the laboratory to a coccolith formed in nature. (See the figures at right.) The synthetic form has a simple rhombohedral shape (a prism with six faces, each a rhombus) and tends to grow randomly with no preferred orientation. In contrast, the coccolith exhibits a very organized, repeating crystal pattern.

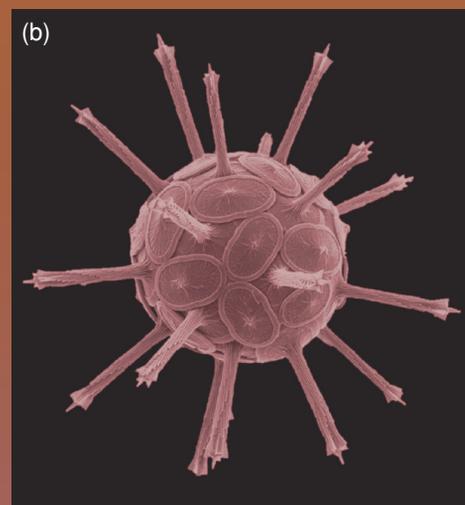
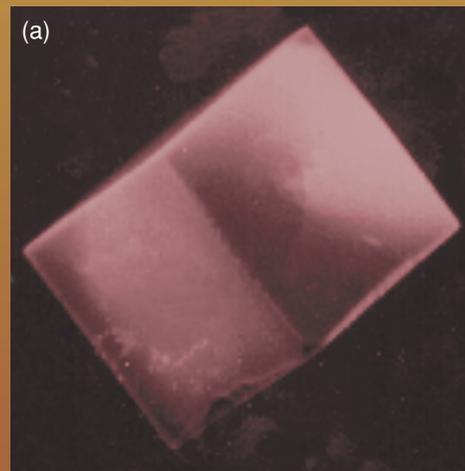
In an effort funded originally by the Laboratory Directed Research and Development (LDRD) Program and now by the Department of Energy (DOE), a Livermore team is using advanced microscopy techniques and molecular

modeling to investigate the effects of interactions between biomolecules and calcium carbonate surfaces. The Livermore team, which includes physicists Jim De Yoreo, Roger Qiu, and Chris Orme, is collaborating with geochemist Patricia Dove of Virginia Polytechnic Institute, molecular biologist Daniel Morse of the University of California at Santa Barbara, biochemist John Evans of New York University, and theoretical physicist Andrzej Wierzbicki of the University of South Alabama.

The multidisciplinary collaboration is studying calcium carbonate because calcium-bearing minerals comprise about 50 percent of all known biominerals. The vast deposits of calcium carbonate laid down by marine organisms are the largest terrestrial reservoir of carbon and hold a historical record of the interplay between Earth systems and biological organisms stretching back to the Cambrian period. Moreover, the richness of calcium carbonate architectures suggests that the mineral can serve as an excellent model for determining the physical mechanisms that underlie biomineralization.

The Many Faces of Crystals

More than 300 identified crystal forms of calcite can combine to produce at least a thousand different crystal variations. Calcite, in its simplest form, grows as a rhombohedron. Similar to other solution-grown crystals, calcite grows when molecules land on the surface of a crystal seed and attach to the edge of a one-molecule-thick layer, called a step. The addition of molecules causes the layer to spread outward until it covers the face of the crystal. The layers form because the crystal contains defects, called dislocations.



(a) The simple shape of a synthetic calcite crystal is contrasted with (b) the complex and organized shape of a coccolith formed in nature. (This coccolith image and the other crystal images in the background at left are courtesy of Markus Greisen and Jeremy Young, copyright Natural History Museum, London.)

A dislocation will naturally generate a step at the surface of the crystal lattice. Because this step never goes away, as the layer grows outward, a spiral ramp of new layers, called a dislocation hillock, forms. Molecules continually adsorb and desorb from the step edges of these hillocks. If the concentration of molecules in the solution is high, the rate of adsorption will be greater than the rate of desorption, and the crystal will grow. If the reverse is true, the crystal will dissolve.

In the past, theories of solution crystal growth were based on the limited capabilities of imaging equipment such as the scanning electron microscope (SEM). The SEM allowed researchers to observe only one or two faces of a crystal and did not allow them to observe the growth mechanisms in real time and at the necessary size scales. More recently, the Livermore team was among the pioneers to use atomic force microscopy (AFM) for investigating the growth of crystals from solutions. AFM enables researchers to image and measure subnanometer changes on the surfaces of every face of a crystal in real time. (See the box on p. 7.) In 2001, the team received a Laboratory Science and Technology Award for its use of this technology in investigating the physical controls on biomineral growth.

To understand the growth mechanisms involved in biomineralization, researchers must know the orientation of a crystal's molecules and how they bond to each other. The molecules in a crystalline structure are arranged in a framework called a crystal lattice. The geometric and chemical relationships between the crystal lattice and organic modifiers, such as proteins, determine the interaction energy between modifier and crystal.

Because crystals found in biomineral structures often exhibit unusual faces not found in synthetic crystals, researchers originally thought that the addition of a modifier must lower the energy of those new faces, allowing the crystal to express a shape that wouldn't have otherwise been stable. "We discovered this theory was incorrect," says De Yoreo, who initiated the Livermore effort. "To understand how a particular additive affects crystal shape, we need to look at how it interacts at the growing steps of the crystal."

A modifier alters the shape and growth rate of a crystal by blocking the growth of certain steps or by increasing the rate at which new molecules attach to steps. Because the geometric and chemical relationships between modifier and crystal are different for each type of step, new crystal shapes can be generated. To

understand the change in crystal shape, the team performed calculations that predicted which steps the modifier would bind to most strongly.

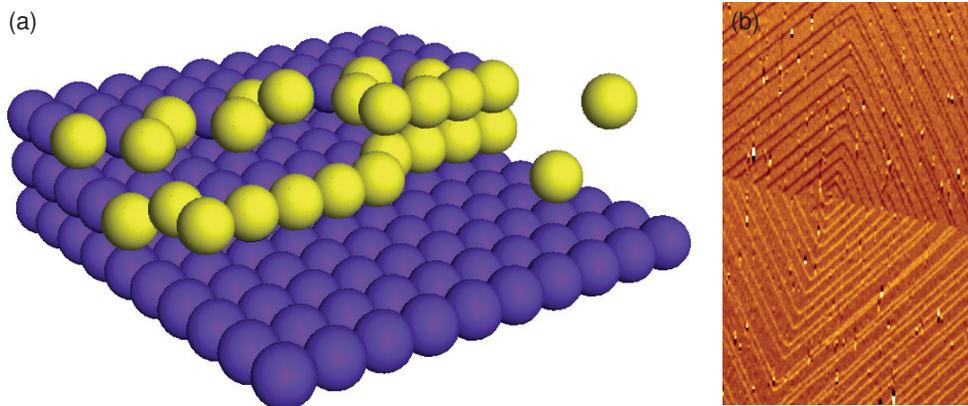
Proteins Modify Growth

In 2005, the team studied how peptide sequences from proteins of abalone and oyster shells—AP7-N, AP24-N, and n16-N—affect calcite growth. Using AFM, the team explored the adsorption of these proteins. Results showed that AP7-N and AP24-N inhibit growth at some of the calcite crystal's steps and accelerate growth in others, resulting in round dislocation hillocks. In contrast, n16-N interacts with corner sites and promotes the emergence of a new set of steps.

To test the importance of protein sequence on the structure of the three proteins, the team scrambled the sequence of peptides in the chain. The scrambled proteins no longer accelerated the step kinetics, illustrating directly that the structure, not simply the chemistry, is important for the effect on crystallization. This study is one of the first to systematically change protein sequence and use AFM to cross-correlate sequence and structure. The process provides a mechanistic understanding of the effects on crystal growth.

In another study funded by DOE, the team investigated whether the most acidic abalone nacre protein, AP8, alters calcite growth and whether differences in control mechanisms existed. The change in growth shape was similar to that seen with small molecules, but surprisingly, the team found that the molecular-scale kinetics are significantly accelerated by AP8. Moreover, although AP8 proteins are much larger than atomic steps, they modify the growth by step-specific interactions. The observed rounding of the step edges and accelerated kinetics indicate that these proteins act as surface-active agents to promote ion attachment at the calcite step.

Qiu says, "The significant and exciting outcome of this work is that we can define a new role for biomolecules in controlling



(a) A crystal grows when molecules land on the surface of a crystal seed and attach to the edge of a layer, called a step. (b) An atomic force microscope image of a pure calcite sample shows that as the molecules attach to a step, the layer spreads, forming a dislocation spiral, or hillock.

crystal growth. Specifically, acidic proteins promote crystallization, which is in stark contrast to an inhibitor of crystallization—a role that is generally postulated and observed for peptides and other small molecules.” The results of the study also create a coherent picture of how protein-induced morphological changes at the molecular scale can guide the growth of a crystal to its macroscopic form.

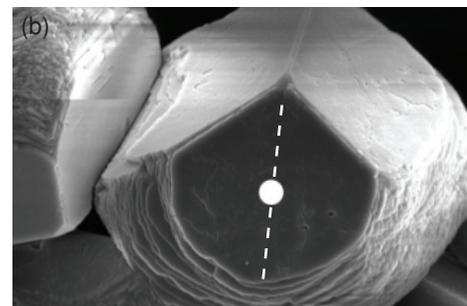
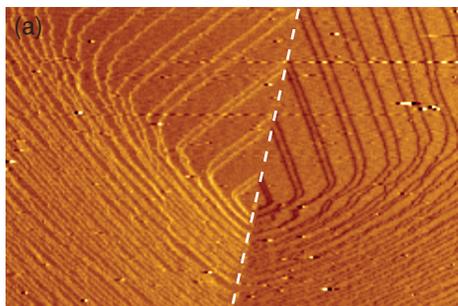
Molecular models confirm that the step edges are the most favorable binding environment for the modifiers. The steps provide the greatest opportunity for the three-dimensional modifiers to make the largest number of bonds. De Yoreo says, “Although the mechanisms of growth modification are diverse, crystal shape is most controlled by the interactions that occur at the step edges of a crystal’s faces. By knowing which steps the modifiers bind to most strongly, we can predict how they change the crystal shape.” These findings are significant for applications in materials

science, bioengineering, geosciences, and medical sciences.

Inhibiting Kidney Stones

Not all biomaterialization processes are aimed at growing crystalline structures. Many organisms rely on arresting crystal growth in order to survive. For example,

arctic fish must live in subfreezing environments without developing ice crystals in their blood. If it were not for the inhibitory effect of certain proteins, the supersaturation of calcium phosphates and oxalates in human blood and urine would be enough to turn a human into a proverbial pillar of salt. Understanding how proteins



(a) An atomic force microscope image shows that the AP8 protein alters the step morphology of a growing calcite crystal by accelerating the growth kinetics. (b) A scanning electron microscope image shows the shape of a calcite crystal grown in the presence of AP8 protein. The morphology in the upper portion remains the same, but the morphology in the lower portion is modified.

Measuring the Topography of Materials

The atomic force microscope (AFM) has been used since 1986 to produce topographic maps of nanostructures. An ultrasharp tip scans across a sample’s surface, and a computer records the path of the tip, slowly building a three-dimensional image. When force and displacement data are combined in various algorithms, the resulting calculations reveal information on mechanical properties, such as hardness, and any other reactions to an applied force.

Unlike the scanning electron microscope, which provides a two-dimensional image of a sample, the AFM provides a true three-dimensional surface profile. Additionally, samples viewed by AFM do not require special treatment that would destroy the sample. AFMs also do not need to operate in a vacuum environment, as do electron microscopes. The AFM can be used on materials that don’t conduct electricity. The microscope is unique in its ability to measure the mechanical properties of both hard and soft materials. The latter has traditionally been more difficult because soft materials, such as biological tissues, are part solid and part fluid.

Although the AFM produces a smaller image area than electron microscopes (micrometers versus millimeters, respectively), it can image and manipulate matter at the nanoscale, allowing researchers to both study a molecule’s structure and properties and organize molecules into predetermined patterns.



Roger Qiu uses atomic force microscopy to investigate how a naturally occurring protein (osteopontin) can control the growth of a kidney stone.

and other biomolecules—including therapeutic agents—inhibit mineralization is crucial for controlling a variety of disorders.

An example of such a disorder is the formation of stones in the human urinary tract. Through funding from the National Institutes of Health, Livermore's biomineralization team is collaborating with Wierzbicki, physical chemist George Nancollas from State University of New York at Buffalo, and nephrologist John Hoyer from Children's Hospital in Philadelphia to investigate the influence of modifiers in inhibiting the growth of kidney stones.

Kidney stones are composed of up to 95-weight-percent calcium oxalate. Calcium oxalate exists in two forms—calcium oxalate monohydrate (COM) and dihydrate (COD). Although normal human urine contains COM and COD, stone formation is suppressed by a number of protein inhibitors and small molecules such as citrate and magnesium.

The Livermore team combined molecular modeling with AFM to provide the first molecular-scale views of COM modification by two urinary constituents, citrate and the protein osteopontin. The team found that while both molecules inhibit the growth kinetics and modify growth shape, they do so by attacking different faces on the COM

crystals. Citrate has a stronger binding energy on the steps of one face than on the steps of the other faces. The results also suggest that when citrate and osteopontin are added simultaneously, a greater inhibitory effect on COM growth exists than when either is applied alone. The results have significant implications for kidney stone disease therapy.

Qiu says, "We don't completely understand kidney stones. For example, we would like to know how proteins control the nucleation of stones, that is, the growth of new crystals." Qiu wants to create a synthetic cell surface for realistically studying nucleation kinetics and how additives may affect nucleation. "In the case of kidney stones, the problem can be addressed at a few different points along the pathway," he says. "We could address it at the nucleation point or at the transformation of COD to COM. Using a synthetic cell would provide us with the most realistic model to understand the starting point of kidney stone formation."

Applications to Energy and Security

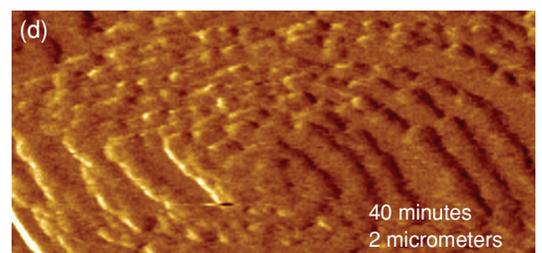
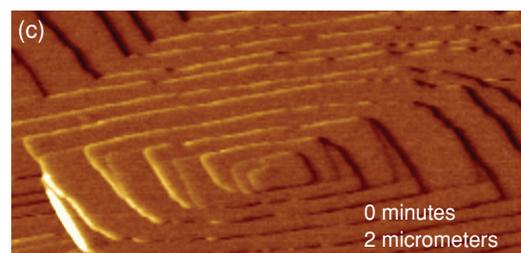
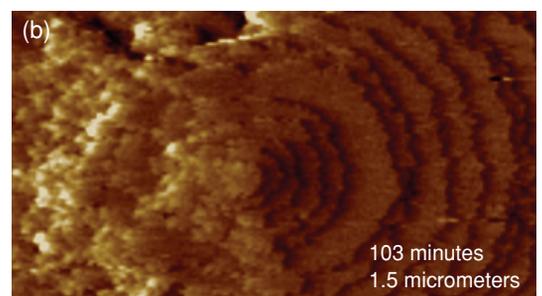
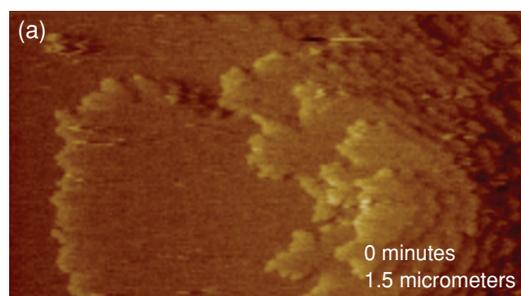
Much of Livermore's early AFM work on crystal growth focused on the need to better understand potassium dihydrogen phosphate (KDP) crystal growth for use on the Laboratory's National Ignition Facility

(NIF), which when completed will be the largest laser and optical instrument ever built. (See *S&TR*, September 2002, pp. 20–29.) In 1983, Novette, one of NIF's predecessors, was the first laser to be engineered with optical frequency converters made of KDP crystals. The crystals convert infrared light at a wavelength of 1,053 nanometers to a shorter wavelength of 351 nanometers.

For NIF's optics, about 600 large slices of KDP are needed. Using traditional crystal growing methods, it would have taken 15 months to grow that number at the large sizes needed. In the early 1990s, a fast-growing method, pioneered in Russia and perfected at Livermore, produced crystals at the required size in just days. The development team won an R&D 100 Award in 1994 for developing the process that produced high-quality KDP crystals for inertial confinement fusion lasers. Their process had, in only 27 days, produced a KDP crystal measuring 44 centimeters across.

The results of the biomineralization studies conducted by the Livermore team may help NIF researchers in another effort. In inertial confinement fusion, a large amount of laser energy is delivered onto a target containing fusion fuel. The type

Atomic force microscope images show at the molecular scale that citrate and a naturally occurring protein, osteopontin, inhibit the growth and change the shape of calcium oxalate monohydrate by attacking the steps on different faces. While citrate strongly affects the steps on the top face (a–b), osteopontin tackles the steps on the side face of the crystal (c–d).



of target envisioned for NIF ignition experiments (those designed to liberate more energy than the fusion fuel absorbs) is a 2-millimeter-diameter capsule containing deuterium–tritium (D–T) gas surrounded by a solid D–T layer.

The specifications for the solid D–T layer are extremely demanding. Surface roughness and defects in the layer can promote hydrodynamic instabilities. Physicist Bernie Kozioziemski, who is on the team developing D–T layers for the ignition capsules, says, “The capsule needs to have a perfect seam between the D–T solid and the D–T gas inside. Any deviation from a perfect sphere indicates roughness either because the crystals are not smooth or because they are not joining well.”

Solid D–T layers are formed by slowly cooling liquid D–T to its freezing temperature. Even the smallest crack may spread as the liquid is cooled to the required temperature. Growing perfectly uniform D–T layers that are free of defects has been a challenge because of the random orientations in the crystals’ initial growth patterns. The team plans to determine the optimal growth orientation for crystals and then find a way to grow this pattern repeatedly for each NIF ignition experiment. One possible approach is to mimic biological organisms by creating a nanoscale template to form a single seed crystal with a preset orientation in an ignition capsule, and then allow the seed to propagate. Comparing the smoothness of D–T layers grown from different seed crystal orientations would quickly indicate the ideal orientation.

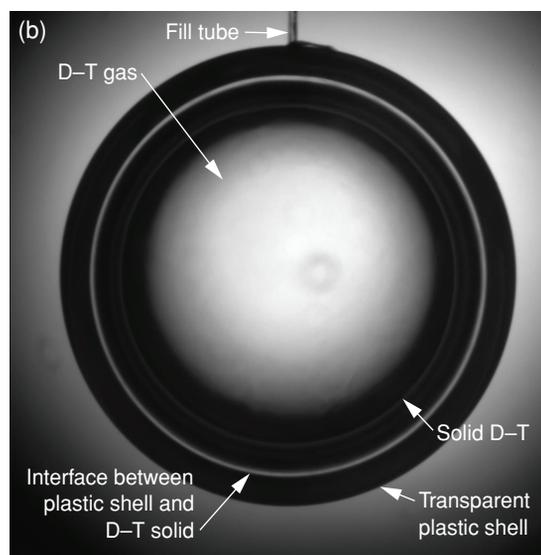
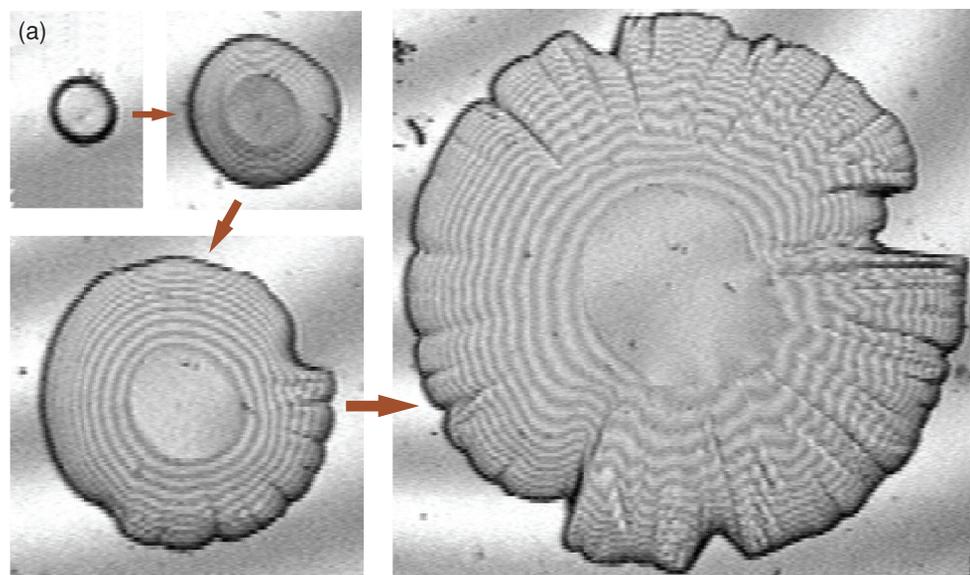
Understanding crystal formation is also important in maintaining high explosives (HE) for stockpile stewardship. Researchers need to know how HE evolves over time. “Many of the powders used in HE may form crystals after a length of time,” says Qiu. “As crystals form, the surface-area-to-volume ratio is decreased, which may affect safety and performance.” Researchers can solve problems with crystal formation in HE by using AFM to examine how HE crystals evolve under different environmental

conditions. Uncovering the formation mechanism will allow researchers to design an effective means to suppress HE crystallization. In addition, researchers can explore the use of compounds that, like modifiers of biominerals, will control the crystallization process.

Mapping Dissolution Mechanisms

Just as molecules most readily attach to the crystal at steps during growth, when molecules leave the crystal during dissolution, they do so more easily by

detaching from the steps, the sites where they have fewer bonds. Understanding dissolution mechanisms has important implications for studies of metal corrosion, a critical issue for DOE’s Yucca Mountain Project. Yucca Mountain, Nevada, is the proposed location to store about 70,000 tons of waste from civilian nuclear power plants and highly radioactive waste from defense-related activities at DOE facilities. Livermore scientists have contributed to the project by characterizing the proposed underground site, determining the effects on the site from



(a) Interferometric images of a growing deuterium–tritium (D–T) crystal show a layer of the crystal that is growing more rapidly than those in the center, leading to a rough surface. (b) Visible light illuminates a transparent plastic shell in which D–T crystals have fused together to form a perfect circle, or interface, between a solid layer of D–T and the shell’s center of D–T gas. Liquid D–T is poured into the fill tube at the top, and the liquid is slowly cooled to form the solid layer.

storing high-temperature radioactive wastes, and selecting and characterizing corrosion-resistant materials for the waste packages. (See *S&TR*, April 2004, pp. 10–16.)

The current repository design calls for waste to be stored in a package consisting of two nested canisters—an outer canister made of a highly corrosion-resistant metal (Alloy 22) and an inner canister made of a tough, nuclear-grade stainless steel (316NG). The proposed outer canister made of Alloy 22 consists of about 60-percent nickel, 22-percent chromium, 13-percent molybdenum, and 3-percent tungsten. Alloy 22 is extremely corrosion resistant at the high temperature and low humidity expected to prevail for hundreds to thousands of years in a repository. However, because it corrodes very slowly, Alloy 22 is difficult to study on laboratory time scales. Therefore, Orme's team conducted studies using Inconel 600, an alloy of nickel–chromium–iron.

To determine how the chemical bonding at the metal surface dictates the initiation of corrosion, Orme and metallurgist Chris Schuh (a former Lawrence Fellow now at the Massachusetts Institute of Technology) combined electron backscatter diffraction (EBSD) with AFM. EBSD can identify the orientation of each crystal grain at the surface of a sample and create a spatial

map of the orientations. After exposing the mapped sample to a corrosive environment, such as diluted hydrochloric acid, the team can then use AFM to determine which orientations dissolve faster than others by measuring the relative heights of different crystallographic orientations. Orme says, “One of the promising aspects of this work is that all facet orientations can be tested simultaneously, making it much easier to identify trends in how materials interact with their environment.”

In the studies with Inconel 600, the team expected the results to show that the metal grains with the fewest number of neighbor bonds at the surface corrode the fastest for the same reason that steps dissolve the fastest. Instead, the low-bonded facets were somehow better protected from dissolution than other facet orientations.

To sort out this puzzle, the team enlisted postdoctoral researchers Jeremy Gray and Bassem El-dasher to carry out a series of experiments, placing Alloy 22 in increasingly corrosive environments. The researchers found that in the most aggressive environments, dissolution occurred as expected, according to the bonding of the metal. However, in less corrosive environments where oxides can form, the order changed. In highly corrosion-resistant

materials, the metal is protected by a very thin layer of oxide, which like paint, provides a barrier between the environment and the metal, preventing it from dissolving. However, unlike paint, the oxide continually reforms if oxygen is present.

Orme says, “The interesting twist here is the competition between metal dissolution and oxide growth. Both processes occur at the most reactive locations on the crystal, and it is a delicate balance to determine which will prevail.” The facets most susceptible to corrosion are also those most able to form a protective coating.

Materials scientists will continue to glean information about the crystal growth process from the fascinating shapes and hierarchical designs of nature's biomineral structures. “We've come a long way in our understanding of crystal growth,” says De Yoreo. “We understand many of the basic mechanisms by which modifiers control growth rate and shape. We also know more about surface science because of these studies. However, everything we've done so far has been in the laboratory. We, admittedly, still understand little about how organisms in nature control nucleation or how they form such remarkable crystalline architectures.”

As Livermore researchers continue to unlock nature's secrets of crystallization, they can look forward to applying this knowledge to many applications in materials science, geochemistry, and medicine and toward the fabrication of new classes of materials.

—Gabriele Rennie

Key Words: abalone nacre, Alloy 22, atomic force microscopy (AFM), biomineralization, calcite, calcium carbonate, calcium oxalate dihydrate (COD), calcium oxalate monohydrate (COM), corrosion, deuterium–tritium (D–T) gas, electron backscatter diffraction (EBSD), potassium dihydrogen phosphate (KDP) crystals.

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(a) A 100-micrometer-square section of an electron backscattered diffraction image of an Inconel crystal shows grain orientations. The colors represent different orientations. (b) An atomic force microscope image of the same area etched with hydrochloric acid shows the susceptibility of each orientation to corrosion. Dark areas in the image indicate orientations that are most susceptible.

